

ALKALI TREATMENT OF Ti6Al4V ALLOY
AND
IT'S EFFECT ON BIO-ACTIVITY

*A thesis submitted in partial fulfilment of the requirement
for the degree of*

MASTER of TECHNOLOGY

In

BIO-MEDICAL ENGINEERING

By

VIRENDRA KUMAR KESARWANI (211BM1210)



Department of Biotechnology and Medical Engineering

National Institute of Technology

Rourkela-769008

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CERTIFICATE

This is to certify that the thesis entitled '**Alkali treatment of Ti6Al4V alloy and it's effect on bio-activity**' submitted by **Virendra Kumar Kesarwani** in partial fulfilment of the requirements for the award of Master of Technology from the Department of Biotechnology and Medical Engineering with specialization in "Biomedical Engineering" at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Supervisor

Place:

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Date:

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Date:

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List of Acronyms:

Ti6Al4V:	Titanium aluminium vanadium alloy
NaOH:	Sodium hydroxide
XRD:	X-ray diffraction
SEM:	Scanning Electron Microscopy
Co-Cr:	Cobalt Chromium
HA:	Hydroxyapatite
PIII	Plasma immersion ion implantation
H ₂ O ₂	Hydrogen peroxide
AHT	Alkali and heat treatment
10M, 700°C	10 molar NaOH treatment and heat treatment at 700°C

ABSTRACT

The purpose of this work was to investigate the bone-like apatite inducement ability of alkali and heat treated Ti-6Al-4V alloy in the HANK solution, with variation of concentration of NaOH solution and heat treatment temperature. The characterization techniques such as Scanning electron microscopy (SEM), X-ray diffraction (XRD), were selected for the evaluation of chemical composition, morphological and crystallinity properties of alkali and subsequent heat treated Ti-6Al-4V alloy. From the XRD analysis it was found that the hydroxyapatite inducement ability increased with the increments of treating temperature, while reduced with increments of concentration.

Keywords: Ti6Al4V alloy; Apatite; alkali treatment.

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION:

The interaction between living bone tissue and implant material has become a prime concern these days because it has an important role in successful implantation for hard tissue replacement. The interaction between living tissue environment and implant completely depends on the surface properties of material. The property of implant to interact with tissue is termed as bioactivity. Bioactivity is defined as : “A bioactive material is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material”(1). Thus an implant material for hard tissue replacement should have appropriate mechanical properties with bioactive surface.

Titanium and its alloys are widely used as implant material, especially in hard tissue replacement for orthopedic and dental implant applications. Ti6Al4V is now being used as common material for bone implants because of its low density, excellent biocompatibility, high strength-to-weight ratio, corrosion resistance and good fatigue strength etc. Besides all these properties, Ti6Al4V alloy has some limitation such as it is a bioinert material and cannot bond directly to living bone, as the bioactive material can, such as Bioglass, hydroxyapatite, and glass ceramics. It has been investigated that titanium implants generally encapsulated after implantation into the living body by fibrous tissue that isolates them from the surrounding bone (2).

Various strategies have been utilized to improve the bone bonding ability of surface of titanium-based implants. For example, Plasma spraying, sol-gel method, electrophoretic deposition, ion implantation. These techniques used bioactive materials such as Hydroxyapatite, Bioglass as coating material to make bioactive surface of metallic implant materials. These coating techniques also required post sintering to densify coating material and increase adhesion strength between substrate materials and coating material(3). But the post sintering process carried out at very high temperature which leads detrimental effects such as change in chemical composition and crystallinity of coating material that limits their use.

Chemical treatments such as alkaline treatment, H_2O_2 treatment, are other popular techniques which are used to modify the surface of metallic implant materials. These techniques enable the surface of implant material to induce apatite layer in living environment. In this work

alkaline treatment was used to modify surface of Ti6Al4V alloy. This method is very simple and cost effective to modify the surface of metallic implants. 10M, and 20M NaOH alkaline solution were used and subsequent heat treatment were done at 600°C, and 700°C. The apatite inducement ability of samples was investigated.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE:

2.1. Biomaterials:

Materials are intended to interact with biological systems and used in medical applications, for example in constructing implant devices and surgical tools known as biomaterials. Biomaterials include several materials systems such as metals, ceramics and polymers which are used to construct artificial organs or implants to replace failing tissue. Chemical response, mechanical properties and biocompatibility in body fluids and tissue environment are the basic criterion for biomaterials. Corrosion is one of the major problems while choosing implant material because it severely affects the performance and life span of implants. The choice of adequate materials depends upon mechanical properties, chemical composition, and surface properties such as durability and functionality governed by bulk properties of material whereas biological response depends upon the surface properties.

The European Society for Biomaterials presented a set of “**Definitions in Biomaterials**” in 1986(4). Which are described below:

Table I: Definitions in Biomaterials

Biomaterial	A non-viable material, used in a medical device, intended to interact with biological systems.
Implant	Any medical device made from one or more materials that is intentionally placed within the body, either totally or partially buried beneath an epithelial surface.
Prosthesis	A device that replaces a limb, organ or tissue of the body.
Artificial organ	A medical device that replaces, in part or in whole, the function of one of the organs of the body.

Biocompatibility is another very important and very essential requirement for any implant material. This shows “the ability of a material to perform with an appropriate host response in a specific application”(5). Biocompatibility is a measure of tolerance of implant material, that living body can withstand without any harmful effect. For the successful application of implant in the human body the chemical and physical properties of material should be very similar with replaced tissue or organ. According to the requirements several materials systems used selectively in medical applications. Which can be classified into three

categories namely metals, ceramics and polymers. For hard tissue replacement it is preferable to choose metallic implant material due to its good mechanical properties. The most commonly used metals for hard tissue replacement are stainless steel, titanium and its alloys and cobalt based alloys.

2.2. Metals:

2.2.1. Stainless steel:

There are many different types of stainless steels are available which are classified on the basis of crystal structures into martensitic, ferritic, austenitic and duplex (austenitic–ferritic) stainless steels(6). The corrosion resistance and mechanical properties of different types of stainless steels differ from each other because these properties depend upon their chemical composition and microstructure. “Cr” is used as alloying element to enhance the corrosion resistance. AISI 316L, single phase austenitic stainless steel, is one of the most popular materials for implant applications (7). To improve tensile and fatigue strength, this alloy is used in a 30% cold-worked state. The compositions of this alloy are 17–19% Cr, 12–14% Ni and 2–3% of Mo. The Mo is used to increase the localized corrosion resistance in chloride-containing environments. Here “L” stands for “low carbon”, which decreases the risk of intergranular corrosion. The elastic modulus of stainless steel is much more (≈ 200 GPa) as compared to bone (≈ 20 GPa). Therefore due to stress shielding effect stainless steel is not suitable for load-bearing bone implants.

2.2.2. Co-Cr-alloys:

Co-Cr alloys are known for their high corrosion resistance property even in chloride environment. Cobalt based alloys are one of the most popular materials for implant application. The alloying elements improve their several attributes for example “Cr” enhance their corrosion resistance property due to formation of an oxide layer enriched with Cr_2O_3 on their surface, while other alloying materials such as nickel, molybdenum improve their mechanical properties and the abrasion resistance. The main problem regarding Cobalt based alloys are fabrication of implants during casting, as solidification during casting may result in large dendritic grains (8)and thus decrease the yield strength of the alloy. Which further lead to, casting defects such as inclusions and micro-pores result in the overall decrease of fatigue strength of the material (9). To avoid these problems Hot-isostatic-pressing (HIP) is the most common technique to densify possible closed porosities in the castings, and homogenization

heat treatments. The forged CoCrMo alloys is a suitable material choice for application encountering strong tribological loads because of high carbon content which improves wear resistance of implant.

2.2.3. Ti and Ti alloy:

Titanium is fourth abundant metal on the earth (~0.86%) after aluminium, iron and magnesium. Therefore titanium and its alloy are easily available and cheaper than other implant materials. Titanium or titanium alloys possess biocompatibility and accepted by the body environment because of their passive and inert oxide layer formed on its surface. This passive layer provides protection against the aggressive environment in body and responsible for higher corrosion resistance. Therefore Ti and its alloys are suitable for medical implants but these are bioinert and hence they do not easily form chemical bonds with host tissue. Thus in order to improve the bio-activity of the surface for direct bonding to surrounding tissue, various methods are applied, for example, to coat a thin layer of bio-active materials such as hydroxyapatite, Bioglass and glass-ceramic.

2.3. Ceramics:

Ceramics are compounds of metallic and non metallic materials with interatomic bonding as ionic and covalent which are generally formed at very high temperature. Ceramics are very popular in the field of hard tissue replacement due to their properties which are very similar to human bone. Ceramic materials that are used in hard tissue repair and various implants commonly referred as bioceramics(10). Bioceramics can be classified further on the basis of their response in living environment such as, bioinert(alumina, zirconia), bioresorbable (tricalcium phosphate), bioactive(hydroxyapatite, bioactive glasses, and glass ceramics)(11). Bioactive ceramics such as hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), and Bioglass($\text{CaO-SiO}_2\text{-P}_2\text{O}_5\text{-Na}_2\text{O}$) are materials of great interest while considering hard tissue applications because of regeneration and bonding with hard and soft tissue in living environment(12).Regeneration and bonding of the hard and soft tissues depend on the compositional ratio of Na_2O , CaO , and SiO_2 .As we know bones are made up of many calcium phosphate salts such as Brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$), and calcium hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$). But hydroxyapatite (HA) is the dominant lattice structure of hard tissue. Therefore, there has been a tremendous interest in using synthetically derived HA for regenerating bone at the defect sites. There are several other properties such as hardness, high

abrasive strength and more resistant towards degradation which makes bioceramics very suitable material for hard tissue repair. But brittleness is major drawback of bioceramics.

2.4. Polymers:

Polymers are organic materials and made up of large number of a repeating unit. The repeating unit is called as a monomer. These monomers are connected with the directional covalent bonds. In biomedical applications polymers generally used for soft tissue replacements. There are various properties of polymers which make it a suitable material for implant applications for example, it can be easily fabricated into various shapes and structures even in case of very complex shapes, the surface properties of polymers can be easily altered. Biopolymers that are used as implant material can be classified into two categories based on their sources, such as natural polymers and synthetic polymers (13). Natural polymers that are used as implant materials generally derived from proteins collagens etc. Polymers are very suitable for those implant application where elasticity is the prime concern for example in cardiovascular applications. There are various limitations too, which restricts its use as implant material for example; Polymers easily absorb water and bio molecules from the surroundings which alters its surface chemistry, poor mechanical properties, leaching problems, and difficulty in the sterilization process (14).

2.5. Chemical surface modifications:

Biological tissue interacts mainly with the first atomic layer of the surface of an implant. Therefore, various techniques have been used to modify surfaces of biomaterials to achieve desired biological responses (15). To optimized tissue interaction, several chemical modifications of implant surfaces have been employed. These methods are used to convert bioinert surface of an implant material, into a bioactive character. Thus direct bone bonding ability of surface should be essential property for implants that are used for hard tissue replacements. Coating of bioactive materials onto the surface of implant are the most direct and popular approach to induce bone bonding ability. Calcium phosphates are known for their bioactivity and their bone binding ability. The first clinical trials on femoral stems with HA coatings were reported by Furlong and Osborn (16) in 1985 and by Geesink (17) in 1986. After that calcium phosphate coatings mainly hydroxyapatite and carbonate apatite have been extensively investigated as bioactive coatings on bioinert implant materials (18). After

implantation of hydroxyapatite coated material, an increased dissolution rate leads to an enhanced release of calcium and phosphate ions which further increase the saturation of body fluids and therefore induces a facilitated precipitation of biological apatite onto the surface of the implant (19). This shows calcium phosphate coated implants are very useful in bone healing process and biological fixation. Various techniques have been employed for calcium phosphate coatings onto metallic implant surface such as plasma spraying, sol-gel coating, electrophoretic deposition, ion implantation and other chemical methods that induce apatite formation after implantation such as alkali treatment and H_2O_2 treatment, in order to associate the mechanical properties of the substrate metals and the biological properties of calcium phosphates. These techniques are described below;

2.5.1. Plasma spraying:

The idea of using plasma spraying to produce HA coatings on endoprosthesis was first proposed in Japan (20). After that plasma spraying technique is the most widely used for orthopedic and dental implant application. It is also use for the surface modification in terms of higher surface roughness. But this technique having various disadvantages such as poor bonding between the plasma sprayed apatite coating and the substrate material due to mismatch of thermal expansion coefficients between two materials, particle release and delamination, which leads to an inflammatory response, and significant changes in the composition and crystallinity of the initial calcium phosphate material, due to the high energy involved in the process. Thus from the above we can conclude that, the structure, composition, and performance of plasma sprayed HA coatings depend strongly on the process parameters such as atmosphere, particle size, plasma gas and flow, input power, distance, substrate angle, cooling rate, etc. thickness of the HA coatings is another big problem because it leads to resorption and poor mechanical property. High temperature process also leads the formation of some amorphous and metastable compounds, such as calcium-deficient hydroxyapatite (CDHA), oxyhydroxyapatite (OHA), calcium oxide (CaO), α -tricalcium phosphate (α -TCP), β -tricalcium phosphate, and tetra calcium phosphate (TTCP) (21). These amorphous and metastable compounds are more soluble than the crystalline HA (22)thereby accelerating fixation of the implant with the bone and promoting bone remodelling and attachment which affects the long term reliability of the implant(23).

2.5.2. Sol-gel method:

The Sol-gel method has various advantages over plasma spraying technique such as, simple and economic method, high chemical homogeneity, fine grain structure and low temperature sintering capability. These properties make it very suitable for the hydroxyapatite coating onto the metal substrate. But, this method also has several complications related to process parameters which affect the chemistry and quality of final products. Brinker and Scherer have broadly divided these parameters into two categories: the respected and the neglected parameters (24). Which are described as following;

Table II: Respected and the neglected parameters in Sol-gel method

S.N.	Respected parameters	Neglected parameters
1.	Choice of precursors	Volatile evolution rate in sol
2.	Concentration of Precursors	Variations in ambient conditions
3.	Water/alkoxidemol ratio	Small impurities in sol
4.	Type and amount of catalyst	Rate of change of viscosity etc.
5.	Control of hydrolysis reaction etc.	

Respected parameters are more important and should give more attention because these parameters define the quality of sol and gel. While neglected parameters should give to lesser attention as compared to previous one. In this method coatings are prepared by simply dipping the substrate in calcium and phosphorous gels(25). As formed coatings show porous, less dense structures and are reported to show a poor adhesion to the substrate material (26). In order to enhance adhesion between coating and substrate material and to increase the density of coating sintering must be done. But due to low temperature sintering capability, possibility to tailor the microstructures and crystallinity of coating material minimizes.

2.5.3. Electrophoretic deposition:

Electrophoretic deposition technique involves the deposition of charged powder particles suspended in a liquid medium due to the application of electric field. Electrophoretic deposition can be divided into two types based on the polarity of electrode on which deposition occurs. When charge particles in suspension are positive in nature then deposition will occur on the cathode and the process is called cathodic electrophoretic deposition. Similarly when charge particles in suspension are negative in nature the process is called the anodic electrophoretic deposition. This method has been widely employed for various applications such as wear resistant coatings, anti-oxidant ceramic coatings, and fabrication of functional films for advanced microelectronic devices and solid oxide fuel cells, and development of novel composites or bioactive coatings for medical implants. Electrophoretic

deposition has various advantages over other coating methods such as deposition can be carried out for any shape of material with a high degree of uniformity, quick, simple and economical process and also enables deposition of complex compounds and ceramic laminates. There are several disadvantages also with this method such that, it cannot use water as the liquid medium, because the application of voltage to water causes the evolution of hydrogen and oxygen gases at the electrodes which could adversely affect the quality of deposits formed, and post sintering is required to obtain the dense and crack free coatings as well as to improve adhesion towards the substrate. Post sintering at elevated temperature further leads to undesirable change in the composition and crystallinity of coating material.

2.5.4. Ion implantation:

Ion implantation is a process in which energetic ions are introduced into the surface of a solid material via bombardment. There are two methods that are used for ion implantation, the first one is conventional beam-line ion implantation and the second one is plasma immersion ion implantation (PIII). First method uses ions that are extracted from an ion source and then accelerated as a collimated and mass-selected beam of high energy, and bombard into the substrate. Raster-scanning is used, either for the ion beam or sample to achieve uniform implantation over a large area. Depending on the size and geometry, work piece allowed for linear translation along one axis or rotation about one or two axes and very sophisticated computer-controlled end stations have been developed to optimize beam-line ion implantation of three-dimensional species. The second method, plasma immersion ion implantation PIII, removes various disadvantages of previous method Such as conformal implantation into objects with complex geometry can be achieved if the plasma sheath conforms to the sample surface. In this method substrate placed directly in a plasma and then pulsed-biased to a high negative potential. A plasma sheath forms around the workpiece and ions are accelerated across the plasma sheath normal to the workpiece surface. So, ions bombard the sample surfaces perpendicularly provided that the plasma sheath dimensions are small compared to the workpiece feature sizes.

Ion beam implantation method has widely used in surface modification of biomaterials such as wear resistance and bone conductivity can be improved by nitrogen and calcium ion implantation, respectively(27). For the calcium ion implantation both methods have been used. Hanawa et al.(28) revealed that the bone conductivity of titanium was

improved by calcium ion implantation as calcium ion implantation accelerates calcium phosphate precipitation on titanium.

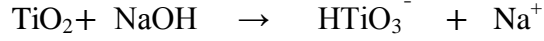
2.5.5. Hydrogen peroxide treatment:

Hydrogen peroxide treatment method is one of the methods that are employed for the improvement of bioactivity of titanium implant. Titania gels can induce the formation of apatite when soaked in a simulated body fluid(29). When H_2O_2 reacts with Ti, a layer of amorphous titania gel forms on the Ti surface. The thickness of the titania gel layer depends almost linearly on the duration of the chemical treatment. Pan et al.(30)suggested that the oxide had a two-layer structure consisting of a thin (<5 nm) and dense inner oxide and an outer porous layer. After hydrogen peroxide treatment subsequent heat treatment above 300°C gradually transformed the gel from the amorphous to crystalline state. If the treatment temperature was below 600°C amorphous gel layer transformed mainly to anatase while the rutile phase was dominant above 700°C(31).

2.5.6. Alkali treatment:

Kim et al. (32)first introduced alkali and heat treatment to enhance the bioactivity of implant materials. This method facilitate the formation of a biologically active bone-like apatite layer on the surface of bioactive ceramics, such as Bioglass(33), hydroxyapatite and glass–ceramic (34).Although hydroxyapatite rapidly bonds to bone, but due to its poor mechanical properties it cannot be use in many orthopaedic applications. Thus In order to create an implant with superior mechanical properties and excellent bioactivity, this method was used to produce bioactive Ti6Al4V. The alkali and heat treatment can be described as follows. The materials are first immersed in a 5–10 M NaOH or KOH solution for 24 h, and then washed with distilled water and ultrasonic cleaning for 5 min. The samples are then dried at 40°Cfor 24 h and finally heated to around 600–800°Cfor 1h.After the heat treatment titanium has been soaked in SBF for 4 weeks, bone-like apatite forms on the surface indicating that the alkali- and heat-treated (AHT) titanium possesses good bioactivity. The reaction involved during alkali and heat treatment and apatite formation in simulated bodyfluids are described as follows:

During the alkali treatment, the TiO_2 layer partially dissolves in the alkaline solution because of the attack by hydroxyl groups.



This reaction is assumed to proceed simultaneously with hydration of titanium.



A further hydroxyl attack on the hydrated TiO_2 produces negatively charged hydrates on the surface of the substrates as follows:



These negatively charged species combine with the alkali ions in the aqueous solution to produce an alkaline titanate hydro gel layer. During heat treatment, the hydrogel layer is dehydrated and converted to crystalline alkali titanate layer. After immersion in SBF solution Na^+ ions from the amorphous layer exchanged by H_3O^+ ions from the surrounding fluid resulting in Ti-OH layer, which is negatively charged and hence, combine selectively with the positively charged Ca^{2+} ions in the fluid to form calcium titanate. As the calcium ions accumulate on the surface, the surface gradually gains an overall positive charge. As a result, the positively charged surface combines with negatively charged phosphate ions to form amorphous calcium phosphate. The calcium phosphate spontaneously transforms into apatite because apatite is the stable phase in the body environment (35).

CHAPTER 3

OBJECTIVE OF PROJECT

3. OBJECTIVE OF PROJECT:

In the present study the hydroxyapatite inducement ability of the Ti6Al4V substrate has been carried out by varying some parameters of alkaline treatment. The variations in concentration of alkaline solution as well as subsequent heat treatment temperature have been done to improve the bioactivity of the surface. The main aim of this experiment is to optimize the bioactivity of alkali treated titanium samples. Alkaline method is very simple and inexpensive method to improve the bio activity of the titanium implants.

CHAPTER 4

MATERIAL AND METHOD

4. MATERIAL AND METHOD:

4.1. Experimental procedure:

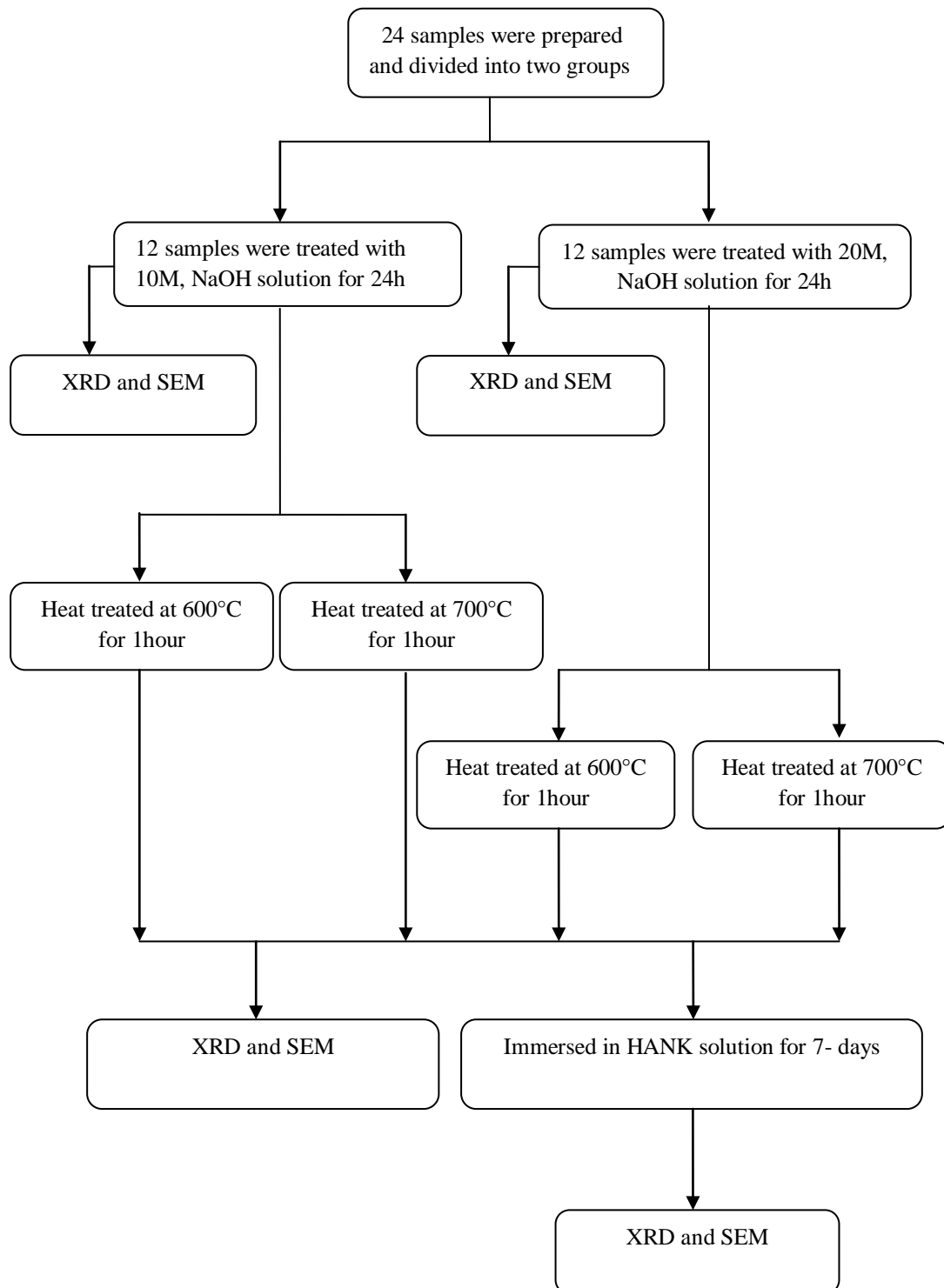


Figure1. Block diagram of experimental procedure.

4.2. Preparation of samples:

4.2.1. Cutting:

Ti6Al4V samples were cut from thin plates with dimensions of around 15mm×10mm×3mm. Hand hacksaw was used as a cutting tool to cut the samples.

4.2.2. Grinding:

Grinding was done on belt grinder to make smooth edges of samples.

4.2.3. Paper polishing:

Samples were polished using three types of emery paper (1/0, 2/0, 3/0). These papers have abrasive particles on their surface. The roughness of emery papers decrease as we go from 1/0 to 3/0. Effective polishing was achieved by using two consecutive emery papers in the perpendicular fashion on the Ti samples. Paper Polishing was mainly done to remove the particle roughness of the titanium samples.

4.2.4. Cloth polishing:

Cloth polishing was done after paper polishing. Cloth polishing was done on a nylon pad on a cloth polishing wheel. This cloth polishing is also called buffing; this is used for only finishing the polishing process. This cloth polishing was done for removing the lines that remained after paper polishing.

4.2.5. Diamond polishing:

Diamond polishing was done after cloth polishing. Diamond polishing was done only for removing very light scratches that remained after cloth polishing. In, this procedure, diamond paste was used along with Hi-Fin spray. This was the final finishing step of polishing of the sample. Finally, a mirror-like surface was produced after diamond polishing was done.

4.3. Alkali treatment:

10M and 20M NaOH solution was prepared and samples were immersed in solution for 24 h, and then washed with distilled water for 5 min. After that the specimens were dried for 24 h at room temperature.

4.4. Heat treatment:

After alkali treatment the samples were heat treated in a muffle furnace in the presence of air. Heat treatment was done in two groups. The first group of samples treated for 1hour at 600°C, while a second group of samples were treated for 1hour at 700°C. During heat treatment, the temperature was gradually increased from room temperature, about 15°C/minute to final temperature and hold final temperature for one hour and then samples were furnace cooling.

4.5. Preparation of HANK solution:

Hank's balanced salt solution was designed for used with cell maintained in non-CO₂ atmospheric conditions. Compositions of hank solution are as follows:

Table III: Chemical composition of HANK solution.

S.N.	Inorganic salts	Chemical formula	Quantity (mg/l)
1.	Calcium chloride dehydrate	CaCl ₂ .2H ₂ O	185.400
2.	Magnesium sulphate heptahydrate	MgSO ₄ .7H ₂ O	200.000
3.	Potassium chloride	KCl	400.000
4.	Potassium phosphate dibasic anhydrous	K ₂ HPO ₄	60.000
5.	Sodium bicarbonate	NaHCO ₃	350.000
6.	Sodium chloride	NaCl	8000.000
7.	Disodium hydrogen phosphate dihydrate	Na ₂ HPO ₄ .2H ₂ O	90.000
8.	Dextrose anhydrous	C ₆ H ₁₂ O ₆	1000.000

4.6. Immersion of samples into HANK solution:

Four samples (10M 600°C, 10M 700°C, 20M 600°C, and 20M 700°C) were immersed into HANK solution for seven days at room temperature.

4.7. Characterization of samples:

Alkali and heat treated samples were characterized by X-Ray diffraction (XRD), and scanning electron microscopy (SEM).

X-Ray Diffraction (XRD):

X-ray diffraction (XRD) is a most powerful technique that extracts detailed information about the chemical composition and crystallographic structure of materials. The X-ray diffraction patterns of alkali and heat treated Ti6Al4V samples at various temperature were recorded on a Philips Analytical Ltd, Holland (PW3040) using Ni-filtered CuK α radiation($\lambda = 1.5406\text{\AA}$) at 40 kV and 30mA. The range for XRD characterization was selected as 20-80 degree with a scan speed of 3 degrees per minute.

Scanning Electron Microscopy (SEM):

A scanning electron microscopy (SEM) is used for complete information about the surface morphology of the sample. SEM characterization of alkali and heat treated Ti6Al4V samples were performed using JEOL JSM-5300 microscope (acceleration voltage 15 and 20 kV). The metallic samples mounted in the SEMs sample holder using graphite impregnated adhesive conductive black 'carbon tape'. SEM was done at various magnifications for example 500X, 2500X, 5000X.

CHAPTER 5

RESULT AND DISCUSSION

5. RESULT AND DISCUSSION:

Alkali and subsequent heat treated Ti6Al4V samples, at various alkaline concentrations (10M and 20M NaOH) and heat treatment temperatures (600°C and 700°C) have been analysed before soaking and after soaking these samples in HANK solution for 7-days. Analysis of these samples is done on the basis of surface morphology with the help of Scanning Electron Microscopy (SEM), and possibility of various compounds on the surface of these samples has been identified with the help of X-ray diffraction (XRD).

5.1. SEM analysis:

5.1.1. 10M, 600°C, treated samples:

SEM images of 10M, 600°C, treated samples, before immersion in HANK solution, and after immersion in HANK solution for 7-days are, shown below. From the images it is clearly seen

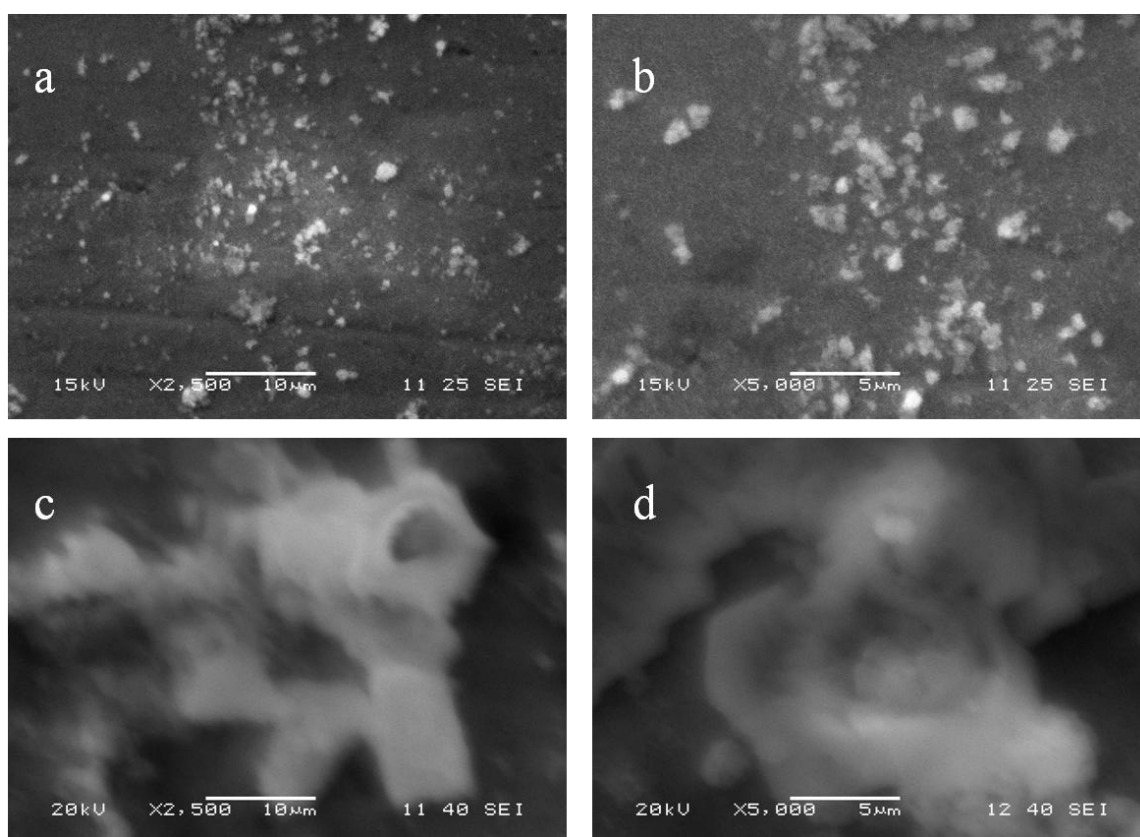


Figure2. SEM images of 10M, 600°C treated sample: (a, b) before immersion in HANK solution, and (c, d) after immersion in HANK solution for 7-days, at two different magnifications.

that the surface morphology of samples get changed significantly, from before immersion in HANK solution to after immersion in HANK solution. The samples before immersion in HANK solution shows small white particle at the surface which might be sodium titanium compounds get converted into large particles, after immersion in HANK solution. These large particles might be hydroxyapatite, and some other calcium phosphorous based compounds. From the images it is clearly seen that the compounds are deposited in agglomerated form, before and after immersion in HANK solution.

5.1.2. 10M, 700°C, treated samples:

SEM images of 10M, 700°C, treated samples, before immersion in HANK solution, and after immersion in HANK solution for 7-days, shown below. The image of sample before

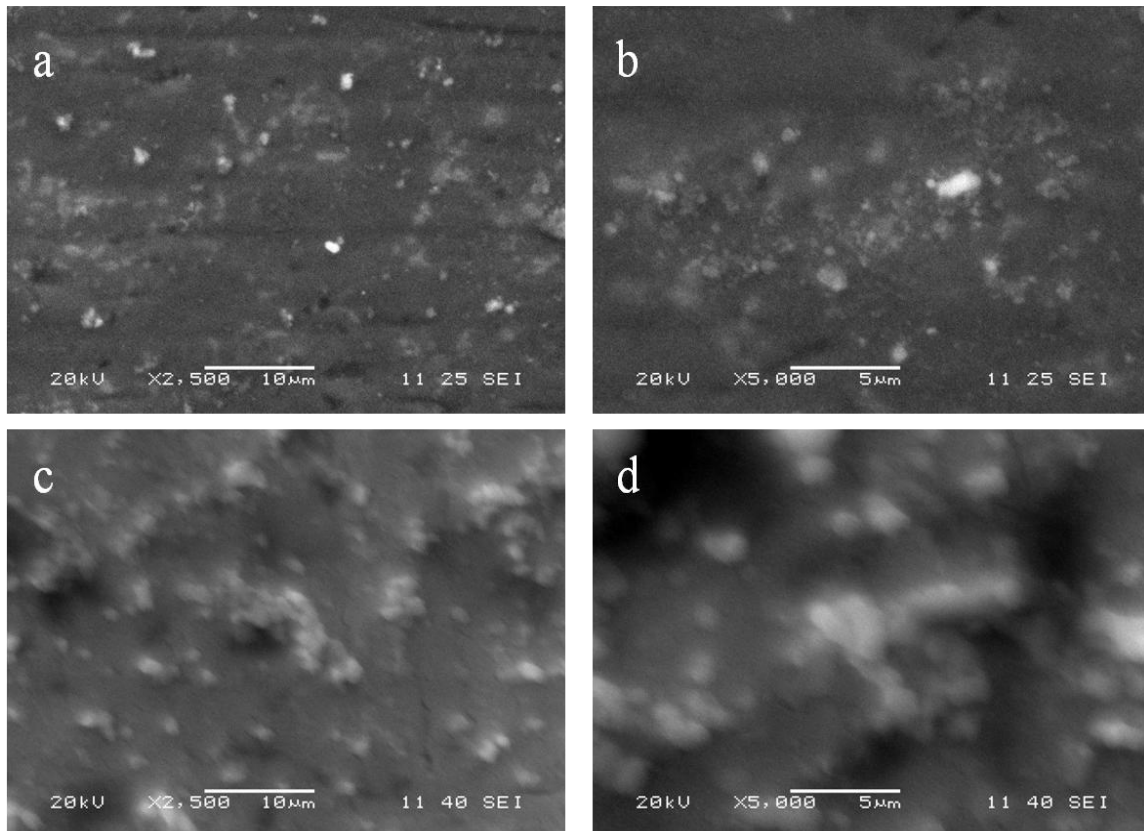


Figure3. SEM images of 10M, 700°C treated sample(a, b) before immersion in HANK solution, and (c, d) after immersion in HANK solution for 7-days, at different magnifications:

immersion in HANK solution is contained very fine white particles, only some particles are in similar size as compared to previous one that is present in 10M, 600°C treated samples.

These particles might be sodium titanium compounds. The image of sample after immersion in HANK solution is contained small round particles which are distributed uniformly throughout the surface, which might be hydroxyapatite, and some other calcium phosphorous based compounds. From the images it is clearly seen that the compounds are uniformly distributed throughout, the surface of samples before and after immersion in HANK solution.

5.1.3. 20M, 600°C, treated samples:

SEM images of 20M, 600°C, treated samples, before immersion in HANK solution, and after immersion in HANK solution for 7-days are shown below. The images of samples before

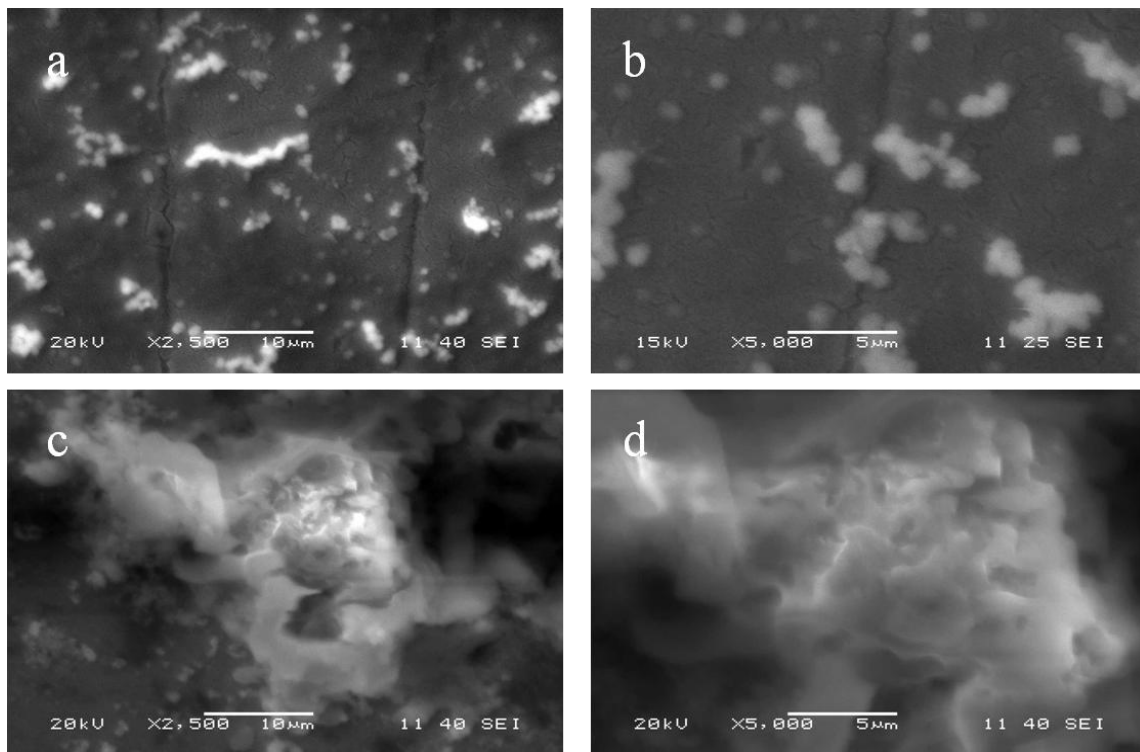


Figure4. SEM image of 20M, 600°C treated sample: (a, b) before immersion in HANK solution, and (c, d) after immersion in HANK solution for 7-days, at different magnifications.

immersion in HANK solution is contained small white particles embedded into the surface layer, which might be sodium titanium compounds, converted into large particles, after immersion in HANK solution, these large particles might be hydroxyapatite, and some other calcium phosphorous based compounds. From the images it is clearly seen that the compounds are in agglomerated form, after immersion in HANK solution.

5.1.4. 20M, 700°C, treated samples:

SEM images of 20M, 700°C, treated samples, after immersion in HANK solution for 7-days, shown below. From the figure it is clearly seen that the white particle size varied from small size to large size, which might be hydroxyapatite, and some other calcium phosphorous based compounds.

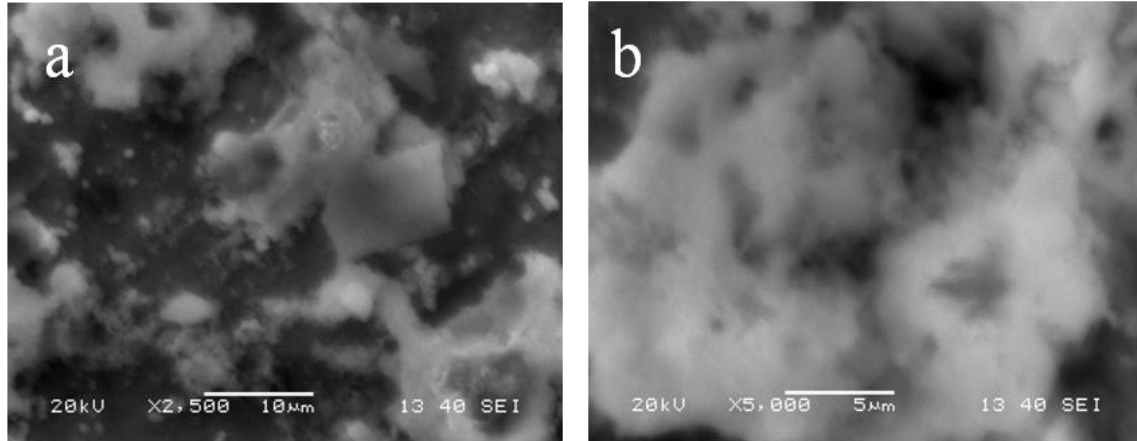


Figure5. SEM images of 20M, 700°C treated sample: (a, b) after immersion in HANK solution for 7-days, at different magnifications.

5.2. XRD analysis:

XRD patterns of 10M and 20M, NaOH treated samples are presented in figure (6) and figure (7). XRD is carried out at various stages of experiment such as, after alkali treatment,

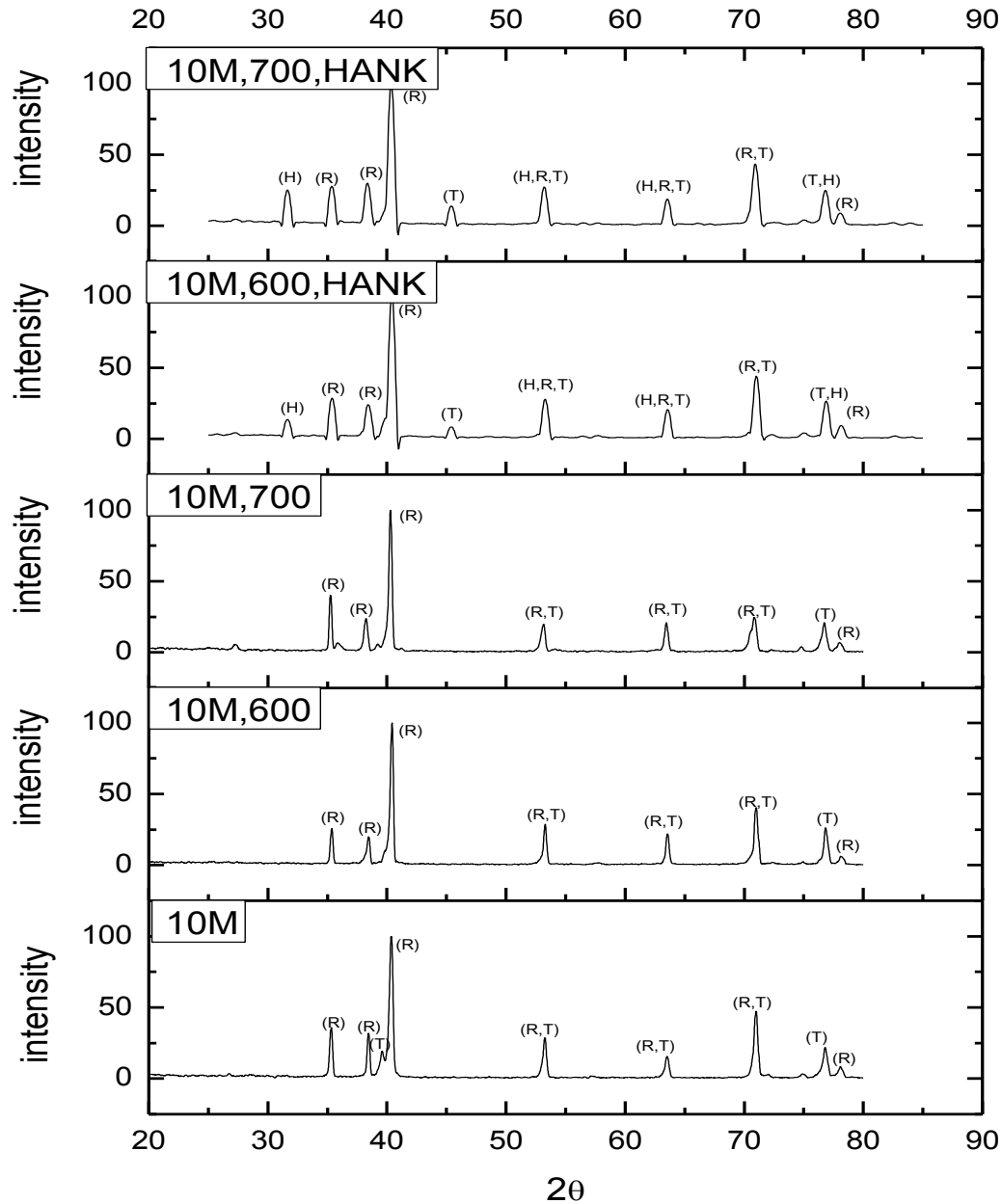


Figure6. XRD of 10M, NaOH treated samples at various stages. (R, rutile H, hydroxyapatite T, titanium oxide).

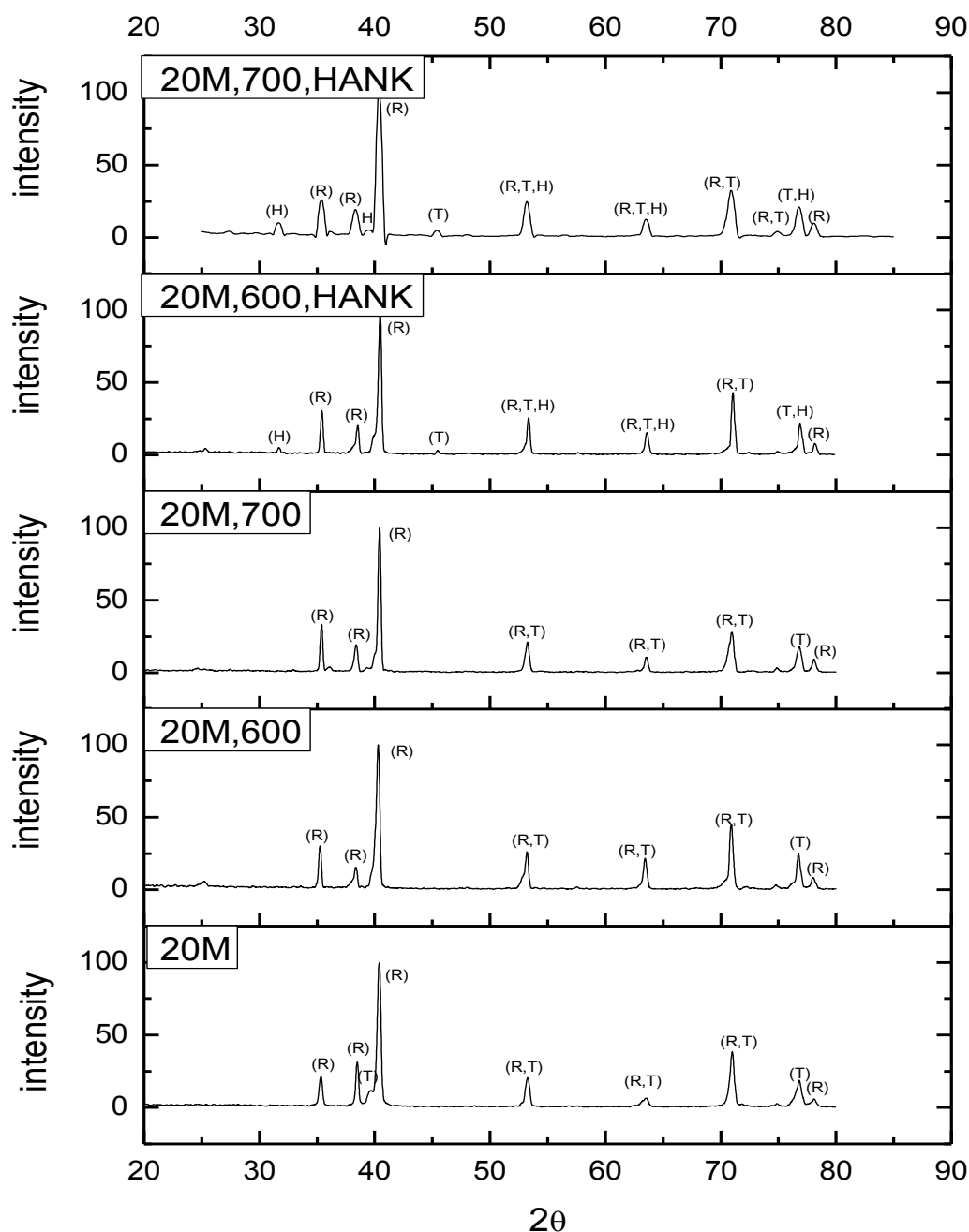


Figure7. XRD of 20M, NaOH treated samples at various stages. (R, rutile H, hydroxyapatite T, titanium oxide).

after subsequent heat treatment, and after immersion of samples in HANK solution. From the XRD peak analysis the presence of rutile, titanium oxides were confirmed at the surface of samples at every stage of experiment. The presence of hydroxyapatite is confirmed in those samples which are immersed in HANK solution. Thus from XRD patterns we can say alkali

and subsequent heat treatment induces apatite formation on the surface of Ti6Al4V alloy when immersed in HANK solution.

It is also found from XRD patterns of the figure (6) and figure (7) that, with increment of temperature, from 600°C to 700°C the peak intensity of hydroxyapatite increased.

From the comparison of XRD pattern of figure (6) and figure (7) it is found that the peak intensity of hydroxyapatite reduced in case of 20M, NaOH treated samples. Thus it is concluded that with the increment of concentration of NaOH from 10M to 20M peak intensity of hydroxyapatite reduced.

From the XRD and SEM analysis, it is found that the hydroxyapatite inducement ability is best for sample which is 10M, NaOH treated and subsequently heat treated at 700°C.

CHAPTER 6

CONCLUSION

6. CONCLUSION:

From the study of XRD, and SEM, of alkali treated samples, the effect of concentration and effect of temperature of subsequent heat treatment are clearly revealed. The effects of variations in these parameters are described as following:

- Presence of hydroxyapatite is clearly found in the samples which are immersed into HANK solution.
- With the increment in temperature from 600°C to 700°C inducement of hydroxyapatite are significantly increases.
- With the increment in concentration from 10M to 20M, inducement of hydroxyapatite is significantly reduces.
- The sample treated with 10M, NaOH solution for 24 hour and subsequent heat treatment at 700°C is found best inducement ability of hydroxyapatite after immersion in HANK solution.
- The sample treated with 20M, NaOH solution for 24 hour and subsequent heat treatment at 600°C is found least inducement ability of hydroxyapatite after immersion in HANK solution.

Future work:

- In the present study the variation of concentration and temperature has been shown and the effect of the inducement of hydroxyapatite on the surface of Ti6Al4V alloy was studied.
- The work may be extended with the biological characterization of the samples including cell adhesion studies by varying some other physio-chemical parameters.
- The parameters may be varied in large range to study the effect of these parameters over the characterization behaviour of the samples and to optimize the bioactivity of Ti6Al4V alloy.

REFERENCES:

1. Cao, W., and Hench, L. L. (1996) Bioactive materials, *Ceramics international* 22, 493-507.
2. Fujibayashi, S., Nakamura, T., Nishiguchi, S., Tamura, J., Uchida, M., Kim, H. M., and Kokubo, T. (2001) Bioactive titanium: Effect of sodium removal on the bone-bonding ability of bioactive titanium prepared by alkali and heat treatment, *Journal of biomedical materials research* 56, 562-570.
3. Ho, W.-F., Lai, C.-H., Hsu, H.-C., and Wu, S.-C. (2010) Surface modification of a Ti-7.5 Mo alloy using NaOH treatment and Bioglass® coating, *Journal of Materials Science: Materials in Medicine* 21, 1479-1488.
4. an einer humanen Knochenmarkzellkultur, H., and Urselmann, F. F. Aus der Orthopädischen Klinik der Heinrich-Heine-Universität Düsseldorf Direktor: Herr Univ.-Prof. Dr. med. Rüdiger Krauspe.
5. Bauer, S., Schmuki, P., Mark, K. v. d., and Park, J. (2012) Engineering Biocompatible Implant Surfaces, *Progress in Materials Science*.
6. Davis, J. (2003) Metallic Materials, Chapter 3, *Handbook of Materials for Medical Devices*, ASM International, Materials park, Ohio, USA.
7. Ratner, B., Hoffman, A. S., Schoen, F., and Lemons, J. E. (2004) Biomaterials science 2 nd edition, Elsevier Academic Press, Amsterdam.
8. Bermingham, M. J., McDonald, S. D., StJohn, D. H., and Dargusch, M. S. (2011) The effect of boron on the refinement of microstructure in cast cobalt alloys, *Journal of Materials Research* 26, 951.
9. Meacock, C., and Vilar, R. (2009) Structure and properties of a biomedical Co-Cr-Mo alloy produced by laser powder microdeposition, *Journal of Laser Applications* 21, 88.
10. Hench, L. L. (1993) *An introduction to bioceramics*, Vol. 1, World Scientific.
11. Hench, L. L. (1991) Bioceramics: from concept to clinic, *Journal of the American Ceramic Society* 74, 1487-1510.
12. Hench, L. L., and Thompson, I. (2010) Twenty-first century challenges for biomaterials, *Journal of The Royal Society Interface* 7, S379-S391.
13. Reichert, W. M., Harris, T. R., Lemons, J., Mikos, A. G., Puleo, D. A., Schoen, F. J., and Temenoff, J. S. (2012) 2011 panel on developing a biomaterials curriculum, *Journal of Biomedical Materials Research Part A* 100, 802-816.
14. Menges, G. (2002) *Werkstoffkunde Kunststoffe*, Hanser Verlag.
15. Sano, K.-I., and Shiba, K. (2003) A hexapeptide motif that electrostatically binds to the surface of titanium, *Journal of the American Chemical Society* 125, 14234-14235.
16. Furlong, R., and Osborn, J. (1991) Fixation of hip prostheses by hydroxyapatite ceramic coatings, *Journal of Bone & Joint Surgery, British Volume* 73, 741-745.
17. D'Antonio, J. A., Capello, W. N., Manley, M. T., and Geesink, R. (2001) Hydroxyapatite femoral stems for total hip arthroplasty: 10-to 13-year followup, *Clinical orthopaedics and related research* 393, 101-111.
18. Rey, C. (1990) Calcium phosphate biomaterials and bone mineral. Differences in composition, structures and properties, *Biomaterials* 11, 13.
19. LeGeros, R. Z., Daculsi, G., and LeGeros, J. P. (2008) Bioactive bioceramics, In *Musculoskeletal Tissue Regeneration*, pp 153-181, Springer.
20. Aoyagi, M., Hayashi, M., Yoshida, Y., and Yao, Y. (1979) Implants for bones, joints and tooth roots, Google Patents.

21. Prev  y, P. S. (2000) X-ray diffraction characterization of crystallinity and phase composition in plasma-sprayed hydroxyapatite coatings, *Journal of thermal spray technology* 9, 369-376.
22. Tsui, Y., Doyle, C., and Clyne, T. (1998) Plasma sprayed hydroxyapatite coatings on titanium substrates Part 1: Mechanical properties and residual stress levels, *Biomaterials* 19, 2015-2029.
23. Nagano, M., Nakamura, T., Kokubo, T., Tanahashi, M., and Ogawa, M. (1996) Differences of bone bonding ability and degradation behaviour< i> in vivo</i> between amorphous calcium phosphate and highly crystalline hydroxyapatite coating, *Biomaterials* 17, 1771-1777.
24. Brinker, C. J., and Scherer, G. W. (1990) *Sol-gel science: the physics and chemistry of sol-gel processing*, Academic Pr.
25. Podbielska, H., and Ulatowska-Jarza, A. (2005) Sol-gel technology for biomedical engineering, *Technical Sciences* 53.
26. Kim, H. W., Kim, H. E., Salih, V., and Knowles, J. C. (2005) Sol-gel-modified titanium with hydroxyapatite thin films and effect on osteoblast-like cell responses, *Journal of Biomedical Materials Research Part A* 74, 294-305.
27. Hanawa, T. (1999) In vivo metallic biomaterials and surface modification, *Materials Science and Engineering: A* 267, 260-266.
28. Singh, R., and Dahotre, N. B. (2007) Corrosion degradation and prevention by surface modification of biometallic materials, *Journal of Materials Science: Materials in Medicine* 18, 725-751.
29. Wang, X.-X., Hayakawa, S., Tsuru, K., and Osaka, A. (2002) Bioactive titania gel layers formed by chemical treatment of Ti substrate with a H< sub> 2</sub> O< sub> 2</sub>/HCl solution, *Biomaterials* 23, 1353-1357.
30. Pan, J., Thierry, D., and Leygraf, C. (1996) Hydrogen peroxide toward enhanced oxide growth on titanium in PBS solution: Blue coloration and clinical relevance, *Journal of biomedical materials research* 30, 393-402.
31. Pan, J., Liao, H., Leygraf, C., Thierry, D., and Li, J. (1998) Variation of oxide films on titanium induced by osteoblast-like cell culture and the influence of an H₂O₂ pretreatment, *Journal of biomedical materials research* 40, 244-256.
32. Kim, H. M., Miyaji, F., Kokubo, T., and Nakamura, T. (1996) Preparation of bioactive Ti and its alloys via simple chemical surface treatment, *Journal of biomedical materials research* 32, 409-417.
33. Kawashita, M., Nakao, M., Minoda, M., Kim, H.-M., Beppu, T., Miyamoto, T., Kokubo, T., and Nakamura, T. (2003) Apatite-forming ability of carboxyl group-containing polymer gels in a simulated body fluid, *Biomaterials* 24, 2477-2484.
34. Kokubo, T., Ito, S., Huang, Z., Hayashi, T., Sakka, S., Kitsugi, T., and Yamamuro, T. (1990) Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W, *Journal of biomedical materials research* 24, 331-343.
35. Tung, M. S. (1998) Calcium phosphate in biological and industrial system, *Dordrecht, MA: Kluwer Academic Publishers*.